Blood pressure phenotypes in relation to the β-adducin *C1797T* polymorphism in the European Project on Genes in Hypertension (EPOGH)

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Methods The association of blood pressure (BP) with the β-adducin *C1797T* polymorphism was investigated in 388 men and 456 women aged 18–60 years recruited from three European populations (Cracow, Poland, $n=300$; Novosibirsk, Russian Federation, $n=274$; Mirano, Italy; $n=270$). Phenotypes included conventional measurements of BP obtained at the second contact with the subjects and 24-h ambulatory BP. Subjects were genotyped for the β-adducin *C1797T* polymorphism. Both a population-based association study and a family-based analysis were performed.

Results Urinary sodium excretion was higher in Cracow than in Mirano (241 versus 185 mmol/day, $P<0.05$) and intermediate in Novosibirsk (206 mmol/day). The β-adducin *T* allele (15.2 versus 9.1%, $P<0.0001$) was more prevalent in Mirano than in the two Slavic centres. In both population-based and family-based association analyses, there was significant heterogeneity between Slavic and Italian subjects in the phenotype–genotype relationships with β-adducin. Adjusted population-based analyses demonstrated that in the two Slavic centres, values of systolic pressure obtained by 24-h, daytime and night-time ambulatory monitoring, but not those measured by conventional sphygmomanometry at home, were significantly higher in *T* allele carriers than in *CC* homozygotes. These results were confirmed in the family-based analysis of offspring using a quantitative transmission disequilibrium test.

Conclusions Phenotype–genotype associations involving blood pressure are influenced by the technique and conditions of the BP measurement as well as by the overall ecogenetic context. *Blood Press Monit* 8:151–154 © 2003 Lippincott Williams & Wilkins.

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Introduction

Blood pressure (BP) is subject to large short-term and circadian variability. The conditions under which it is measured contribute to the overall variability. Indeed, BP can be measured in various surroundings, (e.g. clinic, home and workplace) by differently qualified observers (e.g. physicians, nurses or patients) using different techniques, ranging from the conventional auscultatory technique to the oscillometric approaches implemented in modern automated BP measuring devices. Furthermore, occasional measurements reflect a subject's true BP only to a minor extent, whereas ambulatory monitoring provides BP values throughout the whole day in persons engaged in their usual activities.

BP levels obtained by 24-h ambulatory monitoring, compared with conventional BP readings, demonstrated smaller within-subject reproducibility, are not subject to observer bias or digit preference, and result in a minimization of the white-coat effect [1]. A meta-analysis of nine prospective observational studies [2] also highlighted that in prospective studies outcome is better correlated with a person's usual BP than with BP measurements only obtained at baseline. The latter are

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biased by random fluctuation, so that the true associations with incident diseases are seriously underestimated. This phenomenon – usually termed regression dilution bias – can be avoided by plotting outcome against an unbiased estimate of the usual BP, for instance obtained by BP monitoring. In this way, the slope of the relationship between outcome and BP considerably steepens. A reverse phenomenon may occur when in regression analysis BP is the dependent variable and is plotted against age or body mass index [3].

The determination of the phenotype is of crucial importance in genetic studies. Obviously, BP measurements obtained under varying conditions or using different techniques cannot be considered identical. To what extent the conditions and techniques of BP measurement might influence the phenotype–genotype relationships in genetic studies has not yet been widely investigated. In the present report, as a test case, we studied the association between various BP phenotypes and the β-adducin *G1797T* polymorphism while allowing for important co-variables. Adducin is an ubiquitously expressed membrane-skeleton protein, which consists of either α- and β-subunits or α- and γ-subunits, which to a large extent are similar in amino acid sequence and domain organization [4]. The gene encoding the β-adducin subunit has been localized to chromosome 2p13 and comprises 17 exons [5,6]. Adducin system-wide and at the renal tubular level influences transmembranous sodium transport [4,7,8].

Methods

The epidemiological, genetic and statistical methods used in the multicentre European Project on Genes in Hypertension (EPOGH) are described in detail elsewhere [9,10]. In short, we randomly recruited nuclear families with at least one parent and two siblings from three European countries: 300 subjects from Cracow (Poland), 274 from Novosibirsk (Russian Federation), and 270 from Mirano (Italy). Age ranged from 18 to 60 years.

The participants had their conventional systolic and diastolic (phase V) blood pressures measured in their homes. The observers had to comply with a thorough quality control programme [11]. For the present analysis, we used the average of the five consecutive conventional measurements of BP obtained at the second contact with the subjects. We determined 24-h, daytime and night-time blood pressures from unedited ambulatory recordings. Daytime (10 a.m.–8 p.m.) and night-time (12 p.m.–6 a.m.) were defined according to narrow fixed clock-time intervals [12]. Validated [13,14] oscillometric devices (Takeda A&D TM2430 or SpaceLabs 90207) were programmed to obtain readings at 15-min intervals from 8 a.m. until 10 p.m. and every 30 min for the remainder of the time. Intraindividual means of the ambulatory measurements were weighted by the time interval between successive readings. Standard cuffs had a 12 × 24 cm inflatable portion, but if upper arm circumference exceeded 31 cm, cuffs with a 15 × 35 cm bladder were employed.

We administered a questionnaire to collect information about the subjects' medical history. The participants collected a 24-h urine sample in a wide-neck plastic container. Variation in the β-adducin gene was determined as described elsewhere [9].

Proportions were compared by Fisher's exact test. We first searched for possible co-variables of the phenotypes under study using stepwise multiple regression with the *P*-values for co-variables to enter and stay in the model set at 0.15. Differences between centres were tested with analysis of variance and Tukey's test.

We performed both a population-based association study and a family-based analysis. In the population-based study, continuous traits adjusted for co-variables were first tested by analysis of covariance. In keeping with our previous report on the β-adducin gene [15], we compared *T* allele carriers with *CC* homozygotes. However to allow for non-independence between related subjects, our main analyses were based on generalized estimating equations (GEE) [16]. In the PROC GENMOD procedure of the SAS package (SAS Institute, Cary, North Carolina, USA), we defined the intra-familial correlation matrix as unstructured and we adjusted for co-variables. We tested for heterogeneity across populations and genders using the appropriate interaction terms with the β-adducin genotype.

To take advantage of the family structure, we also performed an analysis of quantitative traits by use of variance components models for family data. We applied the quantitative transmission disequilibrium test implemented in the QTDT program (version 2.3; http://www.well.ox.ac.uk/asthma/QTDT) [17]. With similar adjustment as in the population-based analysis, we investigated the association between phenotypes and allelic transmission using the orthogonal model included in the QTDT package. We also assessed the heterogeneity across populations using maximum likelihood methods as described elsewhere [18].

Results

The sex distribution was similar across centres. Overall, the sample included 388 men and 456 women. The Italian participants were slightly but significantly older (41.8 versus 36.9 years, $P < 0.05$) and had a higher 24-h systolic pressure than the participants of the two other centres (122.4 versus 119.0 mmHg, $P < 0.05$). Home BP was higher in Cracow than in the two other centres

(127.1 versus 124.8 mmHg, $P < 0.05$). In untreated subjects with complete 24-h urine collection [19], the urinary sodium excretion was on average 56 mmol/day higher in Cracow than in Mirano (241 versus 185 mmol/day, $P < 0.05$) and intermediate in Novosibirsk (206 mmol/day).

Within each centre, genotype frequencies of the β-adducin polymorphism did not deviate from Hardy–Weinberg equilibrium ($0.71 < P < 0.84$). Across centres, the β-adducin *T* allele frequency was higher ($P < 0.01$) in Mirano (15.2%) than in Cracow (10.2%) and Novosibirsk (8.0%).

In stepwise regression analysis, sex, age, body mass index, smoking, alcohol intake and use of antihypertensive drugs were significantly and independently associated with the BP phenotypes. In all subsequent statistical analyses, we accounted for these co-variables.

Because allele frequencies were different between the Italian and the two Slavic centres, we first tested for heterogeneity in the phenotype–genotype relationships. In the GEE analysis, we found significant genotype-by-centre interactions, when Mirano was contrasted with the two other centres. *P*-values for the interaction terms indicating heterogeneity were 0.04 for the 24-h systolic pressure, 0.04 for the daytime systolic pressure, and 0.08 for the night-time systolic pressure. However, there was no heterogeneity in the phenotype–genotype relationships between the two Slavic centres. Based on these results, we pooled the Polish and Russian participants and analysed the Italian data separately.

Adjusted population-based analyses demonstrated that in the two Slavic centres, values of systolic pressure obtained by 24-h, daytime and night-time ambulatory monitoring, but not those measured by conventional sphygmomanometry at home, were significantly higher in *T* allele carriers than in *CC* homozygotes (Table 1). For diastolic blood pressure in the two Slavic centres and for all BP components in Italians, the phenotype–genotype relations did not reach statistical significance (Table 1).

The study sample consisted of 261 families (409 parents, 435 offspring). There were 87 families with one offspring and 174 families with two offspring. Our family-based analyses confirmed our population-based association study in that there was significant heterogeneity between the two Slavic populations and the Italians with respect to the association between 24-h systolic BP and transmission of the β-adducin *T* allele ($\chi^2 \geq 23.7$, d.f. 11; $P < 0.01$). We therefore analysed the Slavic and Italian offspring separately. As shown in Table 2, the family-based analyses disclosed associations similar to those observed in the population-based study for the β-adducin *T* allele in relation to systolic BP.

Discussion

The main finding of the present study was that the strength of the phenotype–genotype associations differed according to the BP phenotype (ambulatory versus conventional measurement), the BP component (systolic versus diastolic pressure) and epidemiologic context (Slavic versus Italian participants). Indeed, the association between BP and the β-adducin *1797T* allele was confined to systolic pressure on ambulatory measurement in Slavic participants.

Conventional sphygmomanometry and ambulatory monitoring delineated distinct phenotypes. Trained observers measured the conventional BP in the relaxed home environment after the subjects had rested in the sitting position for at least 5 min. The ambulatory BP is affected by BP variability in response to physical activity, psycho-emotional stress and the different stages of sleep. The conventional BP was the average of five readings, whereas the ambulatory phenotypes reflected a substantially larger number of measurements. Furthermore, whatever the technique of measurement, systolic pressure can generally be more accurately determined than diastolic pressure [20]. Although the results for the conventional systolic BP and those for the conventional and ambulatory diastolic blood pressures did not reach statistical significance in the two Slavic centres, they showed trends which were in line with the findings for the ambulatory systolic BP. Precision of the BP phenotypes depending on

Table 1 Blood pressure phenotypes by β-adducin genotype

	Cracow and Novosibirsk ($n=574$)				Mirano ($n=270$)			
	CC	*CT+TT*	Δ	*P*	*CC*	*CT+TT*	Δ	*P*
24-h systolic (mmHg)	119.7±0.5	122.3±1.1	+2.3 (−5.4; 0.8)	0.03	122.9±0.8	121.8±1.2	−1.1 (−1.4; 4.1)	0.42
24-h diastolic (mmHg)	72.1±0.4	73.2±0.7	+1.0 (−2.4; 0.4)	0.17	73.7±0.5	72.9±0.6	−0.8 (−0.6; 2.6)	0.33
Daytime systolic (mmHg)	126.2±0.6	128.6±1.1	+2.4 (−4.6; −0.2)	0.03	129.4±0.9	128.2±1.3	−1.2 (−1.4; 4.6)	0.43
Daytime diastolic (mmHg)	78.0±0.4	79.2±0.7	+1.2 (−2.8; 0.4)	0.13	78.3±0.5	77.6±0.8	−0.7 (−0.9; 2.6)	0.42
Night-time systolic (mmHg)	109.0±0.5	111.8±1.3	+2.8 (−5.5; −0.2)	0.03	111.1±1.0	110.1±1.5	−1.0 (−2.6; 4.0)	0.59
Night-time diastolic (mmHg)	62.7±0.4	63.8±0.8	+1.1 (−2.8; 0.6)	0.19	65.1±0.6	63.9±0.8	−1.2 (−0.8; 3.1)	0.22
Home systolic (mmHg)	126.0±0.8	128.3±1.5	+2.3 (−5.4; 0.8)	0.14	125.3±1.0	122.6±1.5	−2.7 (−0.8; 6.3)	0.13
Home diastolic (mmHg)	79.9±0.5	80.1±0.8	+0.2 (−1.9; 1.4)	0.77	80.1±0.7	78.6±1.1	−1.5 (−0.8; 3.8)	0.33

Values are arithmetic means±SE. Blood pressure was adjusted for sex, age, body mass index, smoking, alcohol intake and antihypertensive treatment. Δ is the phenotypic difference with 95% confidence interval (CI) calculated by subtracting means in CC homozygotes from means in 179T allele carriers (95% CI in parentheses).

Table 2 Association between systolic pressure and *T* allele transmission

Phenotype	χ^2	*P*
Two Slavic populations		
Informative probands/all sibs (n/n)	83/289	
24-h systolic pressure	3.69	0.05
Daytime systolic pressure	2.50	0.11
Night-time systolic pressure	4.80	0.03
Mirano		
Informative probands/all sibs (n/n)	48/146	
24-h systolic pressure	0.16	0.69
Daytime systolic pressure	0.002	0.97
Night-time systolic pressure	0.35	0.56

The orthogonal model accounted for between- and within-family variability components.The quantitative transmission disequilibrium test were adjusted for sex, age, body mass index, smoking, alcohol intake and antihypertensive treatment.

the conditions of measurement and the number of averaged readings is likely to explain these findings. For this reason, *P*-values should not be considered in isolation, but evaluated within the overall trend in an analysis as reflected by 95% confidence intervals.

Genetic background and environmental factors are important determinants of the association between BP, a complex multigenic trait, and variation in candidate genes, such as β-adducin. The present study showed divergence in the β-adducin *T* allele frequency, which was 8.0% in Russia, 10.2% in Poland and 15.2% in Italy. In a recent Belgian population study [15], the *T* allele frequency was 10.7%. In keeping with published INTERSALT results [21], urinary sodium excretion was approximately 50 mmol/day higher in Cracow than in Mirano and intermediate in Novosibirsk. In the two Slavic centres, but not in Mirano, systolic pressure on ambulatory measurement was on average 2–3 mmHg higher in *T* allele carriers than in *CC* homozygotes. It is therefore possible that the β-adducin *1797T* allele, which is thought to interact with sodium transport [4,7,8], only leads to an increase in BP in the context of a high salt diet as consumed by the two Slavic populations.

In conclusion, phenotype–genotype associations involving BP are influenced by the technique and conditions of BP measurements as well as by the overall ecogenetic context. The frequency of the β-adducin 1719*T* allele differs across European populations. In populations on a high salt intake, presence of the β-adducin 1719*T* may be associated with increased BP. Thus, phenotype–genotype associations involving complex quantitative traits, such as BP, can only be interpreted within their epidemiologic context [22].

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